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MIGRATION PATTERNS OF DENDRITIC CELLS IN THE MOUSE

Traffic from the Blood, and T Cell-dependent and -independent Entry to Lymphoid Tissues

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Lymphoid dendritic cells (DC)[1] (reviewed in references 1, 2) are critical accessory cells for the induction of primary immune responses in vitro (e.g., references 3, 4) and in vivo. For example, administration of DC can trigger host immune responses against antigens (5, 6), induce allograft rejection (7–9), and reverse an Ir-gene defect (10). Donor-derived DC-like cells within nonlymphoid organs could also be important "passenger" leukocytes that directly trigger allograft rejection (7, 8), or host DC may present graft antigens (11). However little is known of the traffic of DC into the tissues.

DC migrate from the tissues via afferent lymphatics as "veiled" cells (12). Some of these cells draining the skin are presumably derived from Langerhans' cells that develop into DC in culture (13). The finding of DC-like cells in most nonlymphoid organs (14) suggests that a major route for DC traffic is from the peripheral tissues to the draining lymph nodes. DC are produced in the bone marrow, but the precursors to DC and DC-like cells in the tissues have not yet been identified. DC are present in peripheral blood (15) where they are similar in phenotype and function to lymphoid DC isolated from spleen (16).

We have developed sensitive techniques to follow the traffic of small numbers of highly purified DC. In this and in an accompanying paper (17) we report on the migration of DC, particularly from the peripheral blood. Here we provide evidence that a major migratory pathway of DC is via the blood circulation and that the traffic of DC into lymphoid tissues is regulated in part by T lymphocytes.

Materials and Methods

Mice

C57BL/10 (C57, H-2^b), BALB/c (BALB, H-2^d), DBA/2 (DBA, H-2^d) and C3H/HeJ (C3H, H-2^k) mice were from Olac Ltd., Bicester, Oxon, United Kingdom. Outbred and inbred BALB nude mice were from Olac Ltd. or Sir William Dunn School of Pathology,

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University of Oxford, respectively. Male animals were recipients of cells prepared from either sex.

Cell Preparation

Mice were killed in ether or by cervical dislocation. DC-enriched preparations and two populations of purified DC were used in these studies.

Unsorted DC. DC were initially prepared from spleens as described (4). Briefly, DC were obtained as low density (<1.08) adherent cells (LODACS) from which many macrophages were removed by readherence to tissue culture plates. These preparations were routinely 70–85% DC enriched and contaminated by 5–10% lymphocytes and 5–15% macrophages. The DC were then purified by two techniques (see below).

Sorted DC. LODACS were incubated at 4°C in the presence of 0.02% sodium azide with saturating concentrations of rat mAbs against mouse macrophages (F4/80) (18), Lyt-1 (53-7.3) (19), Lyt-2 (CD8, 3.155) (20), Fc receptors (2.4G2) (21), and Ig κ chains (187.1) (22), followed by FITC–rabbit anti–rat Ig (Dako Corp., Santa Barbara, CA). Purified DC were obtained by negative selection using a Cytofluorograph System 50 (Ortho Diagnostic Systems Inc., Westwood, MA) interfaced with a 2150 data handler (9); a three-parameter sort was carried out, gating on forward and right angle scatter and fluorescence, at ~3,500 cells/s. The viability and function of the sorted cells are discussed below, and in references 9 and 17. In other experiments, anti–complement receptor type 3 (anti-Mac-1, M1/70) (23) was also included in the first antibody cocktail. Although DC weakly express this molecule it was undetectable under these conditions.

On reanalysis, sorted DC were >99.5% negative for the markers used to remove contaminating cells. They also did not bind mAbs directed against the T cell differentiation antigens CD4 (L3T4) (24) and Thy-1 (25), or B cell markers (RA3-2C2/1; TIB145, and RA3-3A1/6.1; TIB146) (26, 27). However in three separate experiments they were 88, 94, and 98% Ia-positive. Thus, sorted DC contained variable numbers of putative null cells which are considered further in the Results.

EA^- DC. As an alternative to cell sorting (above), Fc receptor–bearing cells (macrophages and some B cells) together with other lymphocytes were removed from LODACS by rosetting with antibody-coated erythrocytes (EA) and a further density fractionation (1). This approach was frequently more convenient than sorting, and the resultant cells were >95% Ia^+.

Other Cells. In some experiments DC were fixed in 0.1% glutaraldehyde for 10 min at room temperature, and washed in serum-containing medium before use. T cells (>95% pure) were derived from high-density (>1.08) nylon-wool nonadherent spleen cells (1).

Cell Labeling and Transfer

A solution of ^{111}In-chloride (Amersham Corp., Amersham U.K.; INS 1P; 370 mBq/ml, sp act 1.85 GBq/μg) was diluted in saline to ~125,000 cpm/μl. Water-soluble carrier tropolone (2-hydroxy-2-4-6-cycloheptatrien-1-on; 4.4 mM in 20 mM Hepes-buffered saline, pH 7.6; Fluorochem Ltd., Derbyshire, U.K.) was mixed in a 2:1 ratio (vol/vol) with this solution for 15 s at 22°C. The mixture of ^{111}In-tropolone was then added to cell suspensions (15 μl/100 μl cells at 10^7/ml; ~1 μCi/10^6 cells) in RPMI 1640 medium containing 5% FCS (Flow Laboratories, Rickmansworth, U.K.) and incubated for 5 min at 22°C (28). The cells were washed, resuspended in serum-free medium, and 2–5 × 10^5 cells were administered intravenously in 0.25 ml via the penile vein, or subcutaneously in 25 μl into each footpad of (ether-) anesthetized recipients. The labeling efficiency was >65% for DC and >45% for T cells; cell viability was >90%, as determined by trypan blue exclusion.

Cell Migration Studies

At various time intervals after cell transfer, the mice were killed by cervical dislocation and exsanguinated. The following organs were removed in their entirety for direct measurement of radioactivity in a well-type gamma counter: liver, spleen, lungs, mesenteric lymph nodes (MLN), peripheral lymph nodes (axillary, cervical, and brachial), Peyer's

patches, small intestine (gut), and both kidneys; a sample of skin with subcutaneous tissue was also taken. Peripheral blood was collected and the radioactivity adjusted to cpm/ml (1 ml is the approximate volume of blood per mouse). In addition, after subcutaneous transfer, the activity of the hindfeet and popliteal, inguinal, and para-aortic lymph nodes was measured; to facilitate identification of the nodes, ~25 μl of 0.5% trypan blue (Flow Laboratories) was injected subcutaneously at least 10 min before harvesting.

The activity in each tissue was expressed in two ways: (*a*) as a percentage of the total radioactivity injected; and (*b*) as a percentage of the total radioactivity recovered per 0.1 g tissue sample (specific activity), which allows for the difference in size of the tissues and the actual amount of label that is accessible.

Statistical Analysis

The significance of the differences between experimental groups were ascertained by Student's *t* test.

Results

Experimental Design. In the first set of experiments the time course of migration of ^{111}In-labeled unsorted DC from the blood into lymphoid and nonlymphoid compartments was established. In the second series, the traffic of sorted DC in syngeneic, allogeneic, splenectomized, and nude recipients was studied. The third series of experiments was designed to trace DC traffic via the afferent lymphatics, after subcutaneous administration to the footpad. Control experiments confirmed that DC function was not altered by radiolabeling (17). The migration patterns of T cells processed from the same spleens as DC were studied for comparison.

Kinetics of Migration of Unsorted DC and T Cells from the Blood. Radiolabeled, unsorted splenic DC (70–85% pure) were administered intravenously into syngeneic mice (Fig. 1). DC were sequestered primarily in the lungs until ~1 h after adoptive transfer, but the radioactivity declined exponentially to minimal levels (<1%) by 24 h. At the same time there was a progressive increase in the numbers of DC entering the liver and spleen, reaching plateau levels (~42% and ~13%, respectively) between 3 and 24 h. Thus, DC do not localize in these sites merely by capillary trapping on their first pass through them. T lymphocytes were initially retained in the lungs until ~30 min and also migrated to liver and spleen, but in contrast to DC ($p < 0.001$), they reached plateau levels of ~15% and ~36%, respectively (Fig. 1).

30–50% of the radioactive label was spontaneously released from ^{111}In-labeled DC that were cultured in vitro over 24 h. Subsequent reutilization of the label by freshly added DC was negligible (<5%). This, and the fact that only a minimal amount of radioactivity was present in the tissues after intravenous administration of an equivalent dose of cell-free ^{111}In (Fig. 1, Table II legend), indicates that the radioactivity detected in vivo reflects cellular migration from the blood and is not due to reutilized isotope that was carried over or released from the cells.

Tissue Localization of Sorted DC. The migration of sorted DC in syngeneic and allogeneic recipients was examined (Table I). Sorted DC, compared with unsorted DC (Fig. 1, and not shown), showed an increased sequestration in the liver (e.g., 57.0% and 41.6%, respectively, $p < 0.001$, for syngeneic strains at 24 h) but a decreased accumulation in the spleen (6.9% and 13.4%, $p < 0.001$). Nevertheless, the spleen was again the principal site of DC localization in terms

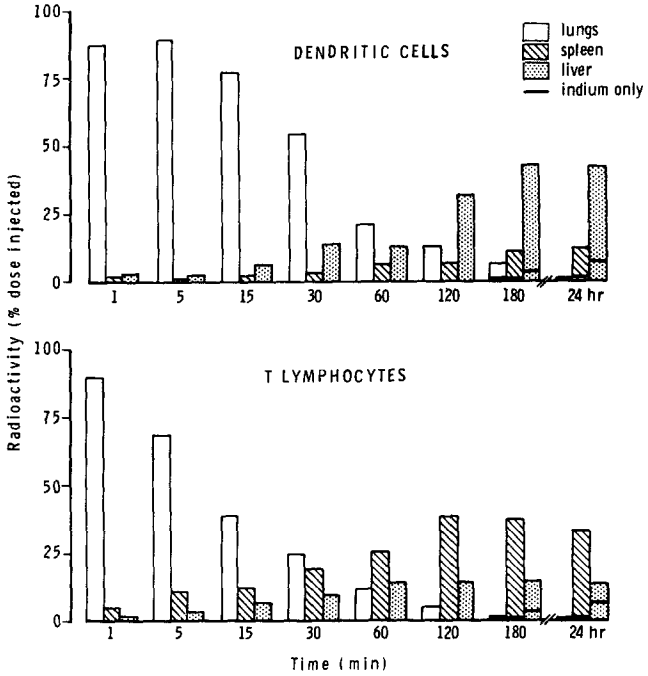

FIGURE 1. Time course of migration of unsorted dendritic cells and T lymphocytes from the blood into the lungs, spleen, and liver of normal mice. 4×10^5 DC (70–85% pure) or T cells (>95% pure) from spleens of BALB mice were labeled with ^{111}In-tropolone and injected intravenously into syngeneic recipients. The radioactivity in the tissues at the indicated times is expressed as a percentage of the total dose injected. The activity at 3 and 24 h after injection of ^{111}In alone is also indicated. Means of three to seven experiments are shown.

of specific activity. The migration of sorted DC to mesenteric and peripheral lymph nodes, and to Peyer's patches was negligible (Table I). In contrast, T lymphocytes homed readily to these lymphoid tissues and relatively small numbers were retained in the liver (Table II). The differences in traffic between sorted and unsorted DC could be explained by the removal of cells with the migratory phenotype of T cells. In contrast to viable DC, glutaraldehyde-fixed DC were preferentially trapped in the lungs of syngeneic recipients and their numbers were reduced in liver and spleen (Table I, legend). The amount of radiolabel in other tissues after intravenous transfer of viable DC was not significantly above that after administration of glutaraldehyde-fixed DC (Table I, and legend). Thus, DC can migrate from peripheral blood into some lymphoid and nonlymphoid compartments, but unlike T cells they are unable to enter lymph nodes.

The migration patterns were unaffected by histocompatibility barriers as only trivial differences were observed between syngeneic and allogeneic recipients (Table I). Nor did they represent the preferential return of splenic DC to their site of origin since DC enriched from mesenteric lymph nodes (1) migrated similarly (data not shown). The in vitro manipulation and purification procedures did not appear to affect the traffic of sorted DC, since the migration of unsorted DC that were preincubated under conditions used for labeling was similar to that of untreated and unsorted DC (i.e., as for Fig. 1).

Despite sorting, some DC preparations contained Ia^- cells (see Materials and Methods). They did not contribute to, or affect the interpretation of, our results. Firstly, when radiolabeled sorted DC were resorted for Ia^+ cells and transferred, the ratio of specific activities in the spleen and liver was 1.42, i.e., within the

TABLE I

Tissue Localization of Sorted Dendritic Cells

Tissue	Radioactivity per organ (percent total dose injected)				Radioactivity per 0.1 g (percent total label recovered)			
	Syngeneic		Allogeneic		Syngeneic		Allogeneic	
	3 h A	24 h B	3 h C	24 h D	3 h	24 h	3 h	24 h
Liver	57.45	57.01	51.73	54.79	7.42	7.39	6.75	7.04
Spleen	6.92	6.93	9.61	8.79	9.46	9.50	13.26	11.93
Lungs	3.37*	0.34§	4.86§	0.55*	3.56	0.36	5.19	0.58
MLN	0.06	0.05	0.06	0.06	0.21	0.17	0.21	0.20
Peyer's	0.02	0.04	0.01	0.02	0.09	0.17	0.04	0.09
Gut	1.24	2.56§	1.66§	2.73	0.22	0.46	0.30	0.48
Skin	2.36	4.41§	1.97§	3.70	0.14	0.26	0.12	0.22
Kidney	3.42	2.96	4.98	5.86‡	1.38	1.20	2.04	2.36
Blood	0.87	0.46§	0.76§	0.38	NA	NA	NA	NA
Total	75.71	74.76	75.64	76.88				
n	3	3	4	4				
p	A/C	A/B	C/D	B/D				

$2–5 \times 10^5$ sorted DC (>99.5% pure) labeled with ^{111}In-tropolone were injected intravenously into syngeneic and allogeneic untreated mice. Lymphoid and nonlymphoid tissues were harvested at 3 h and 24 h for measurement of radioactivity. The activity in each organ was expressed as a percentage of the total dose injected, and as a percentage of the total label recovered per 0.1 g tissue sample. Means of the indicated number of experiments (n) and p values between the columns are footnoted. Blank spaces indicate values that are not significant. NA, not applicable.

Syngeneic strains were: BALB, C57 and DBA; allogeneic combinations were BALB to C3H, C57 to DBA, and C57 to BALB. No significant differences were found between the individual strain combinations within the groups of syngeneic or allogeneic recipients, so the results have been combined.

The activity in peripheral lymph nodes (brachial, cervical, and axillary) was similar to that of MLN (data not shown).

When glutaraldehyde-fixed, ^{111}In-tropolone–labeled DC were adoptively transferred into syngeneic recipients, the percentage of total radioactivity per organ at 3 and 24 h was, respectively: liver, 8.3 and 18.49; spleen, 0.67 and 2.35; lungs, 23.94 and 5.67; MLN, 0.02 and 0.11; Peyer's, 0.03 and 0.06; gut, 1.02 and 2.67; skin, 3.62 and 4.59; kidney, 4.4 and 8.64; and blood, 2.4 and 0.56/ml; ($n = 2$). Most of the radioactivity in the blood was in the plasma rather than the cells, after injection of viable or fixed cells.

* $p < 0.05$.
‡ $p < 0.01$.
§ $p < 0.001$.

range for sorted DC of 1.64 ± 0.27 (mean ± SD, the ratio for T cells being 25.73). Secondly, the resorted Ia$^-$ cells contained <10% of the expected radiolabel, precluding direct testing of their in vivo localization. They may be nonviable cells that were killed or not completely removed by cell sorting.

Migration of DC in Splenectomized Mice. To exclude the possibility that small numbers of DC could enter the lymph nodes from the blood but were undetectable by our techniques, we followed their traffic after splenectomy of the recipients, a procedure that results in increased migration of lymphocytes to lymph nodes (29). Unsorted DC were unable to enter the lymph nodes of splenectomized mice (not shown), and the otherwise spleen-seeking DC were quantitatively diverted to the liver (41.6% and 53.0% in normal and splenecto-

TABLE II
Tissue Localization of T Lymphocytes

Tissue	Radioactivity per organ (percent total dose injected)				Radioactivity per 0.1 g (percent total label recovered)			
	Syngeneic		Allogeneic		Syngeneic		Allogeneic	
	3 h A	24 h B	3 h C	24 h D	3 h	24 h	3 h	24 h
Liver	15.33*	14.71*	21.40*	15.50	2.24	2.12	3.27	2.50
Spleen	37.37‡	34.90	27.06	27.92*	57.63	53.27	43.68	47.57
Lungs	1.56	0.84*	1.76§	0.44‡	1.86	0.99	2.20	0.58
MLN	4.21	6.60‡	5.78	7.52	16.30	25.29	23.42	32.16
Peyer's	3.12	4.66*	2.78	3.55	15.13	22.37	14.12	19.02
Gut	0.55	0.75	0.36‡	0.68	0.11	0.15	0.08	0.15
Skin	1.46	1.98	1.34	1.76	0.10	0.13	0.09	0.13
Kidney	1.74*	1.96	2.58	2.50	0.80	0.89	1.24	1.26
Blood	0.23	0.07§	0.19§	0.06	NA	NA	NA	NA
Total	65.57	65.97	63.25	59.93				
n	6	5	5	5				
p	A/C	A/B	C/D	B/D				

2–5 × 10^5 T cells labeled with ^{111}In-tropolone were injected intravenously into syngeneic and allogeneic mice. Lymphoid and nonlymphoid tissues were harvested 3 and 24 h later for measurement of radioactivity. For details see Table I legend.

When cell-free ^{111}In was administered intravenously, the percentage of total radioactivity per organ at 3 and 24 h was respectively: liver, 3.82 and 7.35; spleen, 0.45 and 1.77; lungs, 0.99 and 0.82; MLN, 0.06 and 0.03; Peyer's, 0.02 and 0.02; gut, 0.22 and 0.18; skin, 0.64 and 0.78; kidney, 1.1 and 1.52; and blood, 25.4 and 8.8/ml (n = 3 or 4).

* $p < 0.05$.
‡ $p < 0.01$.
§ $p < 0.001$.

mized mice, respectively; $n = 4$, $p < 0.01$). In contrast, the migration of T cells to MLN of splenectomized mice rose sharply (6.6% to 19.8%, $p < 0.001$), while the increase in the liver was less dramatic (14.7% and 20.5%, $p < 0.01$). Thus, one of the distinctive features of DC is their inability to migrate from peripheral blood into lymph nodes and hence to recirculate.

Migration of DC from the Blood in Nude Mice. DC can form cell aggregates, or clusters, with lymphocytes in culture in an MHC-independent manner (30). We initially hypothesized that DC might enter the spleen through an analogous interaction in vivo. Therefore we traced radiolabeled unsorted DC after intravenous injection in nude mice that lack mature T cells. At 24 h, the activity within the spleens was greatly reduced to 3.2% (Table III); this value should be compared with 13.4% in the spleens of euthymic mice and 2.4% for glutaraldehyde-fixed cells (Table I, legend). Nude mice were then reconstituted with syngeneic splenocytes or T cells 24 h before DC transfer. The numbers of DC in the spleens of these animals now resembled those in the spleens of euthymic mice (Table III). The numbers of DC entering the livers of nude mice were inversely related to those migrating to the spleen (Table III), as for splenectomized hosts (see above). We conclude that migration of DC from the blood into the spleen is dependent on the presence of T lymphocytes.

Migration of DC via Lymphatics. Having demonstrated that DC do not have

TABLE III

Localization of Unsorted Dendritic Cells in Euthymic, Nude, and Lymphocyte-reconstituted Nude Mice

Tissue	Radioactivity per organ (percent total dose injected)			
	Euthymic A	Nude B	Nude + SPL C	Nude + T D
Liver	41.57*	59.84*	41.97	37.12
Spleen	13.42*	3.21*	9.35‡	10.60‡
MLN	0.06	0.08	0.07	0.07
n	6	4	5	5
p	A/B	B/C	A/C	A/D

Unsorted BALB DC were labeled with ^{111}In-tropolone and injected intravenously into euthymic recipients, nude mice, or nude mice reconstituted 24 h earlier with 10^8 unfractionated splenocytes (SPL) or 4×10^7 T cells. The liver, spleen, and MLN were removed 24 h after injection and the activity in each was measured. There were no differences between the results for outbred and inbred mice, and they have been combined. Means of the indicated number of experiments (*n*) and *p* values between the groups are shown.

* $p < 0.001$.

‡ $p < 0.01$.

access to the lymph nodes from the blood, we followed their traffic via the afferent lymphatics. Radiolabeled EA^- DC (which migrate similarly to sorted DC after intravenous transfer, data not shown) were administered subcutaneously into the footpads of syngeneic mice. As shown in Table IV, a large amount of activity was retained in the footpad until at least 24 h. However, some DC migrated to the draining popliteal lymph nodes as early as 3 h after administration and were present in relatively high numbers at 24 h. Virtually no activity was detected in the inguinal lymph nodes, although a very small amount was found in the para-aortic nodes. T lymphocytes also homed to the popliteal lymph nodes after footpad injection, but at a slower rate compared with DC, and they were retained here for at least 24 h (Table V). In contrast, only background radioactivity was recorded in the popliteal lymph nodes after injection of glutaraldehyde-fixed DC or cell-free isotope (Table V), or in the contralateral nodes when viable DC were administered into one footpad (not shown). Thus, viable DC can enter the draining lymph nodes from the peripheral tissues via the afferent lymphatics.

Since migration of DC from the blood into the spleen is dependent on the presence of T cells (Table III), we also examined migration of DC via the afferent lymphatics in nude mice. There was no difference in the accumulation of DC in the popliteal lymph nodes between euthymic and nude mice (Table IV). Therefore, migration of DC from the peripheral tissues into lymph nodes seems to be independent of the presence of T cells.

Discussion

We have examined the migration patterns of small numbers of purified splenic DC in the mouse, using a technique that was based on the methods originally described by Gowans and Knight (31) for studying the migration of mature rat lymphocytes. ^{111}In-tropolone was used as a radiolabel since it seems to fulfill the

TABLE IV

Tissue Localization of EA⁻ Dendritic Cells Administered into the Footpad of Euthymic and Nude Mice

Tissue	Radioactivity per organ (percent total dose injected)				Radioactivity per 0.1 g (percent total label recovered)			
	Euthymic		Nude		Euthymic		Nude	
	3 h A	24 h B	3 h C	24 h D	3 h	24 h	3 h	24 h
Popliteal LN	0.65	1.18	1.78	1.02	57.95	141.43	86.6	125.9
Inguinal LN	0.05	0.01	0.05	0.01	2.21	0.34	0.67	0
Para-aortic LN	0.06	0.14	0.22	0.1	1.89	9.68	3.59	3.82
MLN	0.03	0.06	0.05	0.01	0.17	0.22	0.16	0
Peyer's	0.003	0.04	0.003	0.06	0.03	0.4	0	0.65
Gut	0.91	2.17*	0.75‡	1.84	0.18	0.52	0.16	0.46
Liver	1.95	4.23‡	2.33	5.7	0.4	0.91	0.38	1.45
Spleen	0.23	0.2	0.35	0.69‡	0.35	0.52	0.39	1.18
Lungs	0.32	0.3	0.33	0.42	0.38	0.42	0.37	0.56
Kidney	2.55	6.04§	1.62‡	5.06	1.76	4.59	1.13	4.27
Skin	2.45	3.4	1.2	2.73	0.27	0.4	0.17	0.61
Hind feet	44.31	27.44*	37.48*	24.89	26.9	18.35	25.43	22.99
Blood	2.3	0.72	3.15	2.11‡	NA	NA	NA	NA
Total	55.81	45.93	49.31	44.64				
n	4	5	3	3				
p	A/C	A/B	C/D	B/D				

DC were prepared by EA rosetting, labeled with ^{111}In-tropolone, and injected subcutaneously into euthymic and nude mice. For other details see Materials and Methods and Table I legend.

* $p < 0.05$.

‡ $p < 0.01$.

§ $p < 0.001$.

criteria proposed for the cell markers used for in vitro tracer studies (32). In contrast to other isotopes, particularly sodium (^{51}Cr) chromate to which DC are particularly "leaky" (our unpublished observations), ^{111}In-tropolone labels very efficiently, attaches firmly to cytoplasmic components, is relatively nontoxic, and is easily detectable in the organs. Furthermore, the cells can be processed in the presence of serum (28), an advantage in DC preparation. The use of ^{111}In-tropolone prevents the formation of ^{111}In-complexes with plasma transferrin, which takes place when ^{111}In-oxine is used (32).

Our results show that DC have a characteristic and distinctive migratory phenotype. In particular, we have demonstrated a major migratory pathway for DC from the blood, and T cell–dependent and –independent regulation of entry of DC to lymphoid tissues.

Migration of DC from the Peripheral Blood. Although DC were initially sequestered in the lungs after adoptive transfer, they subsequently migrated into some lymphoid and nonlymphoid compartments (Fig. 1). The primary site of DC localization in terms of specific activity in both syngeneic and allogeneic recipients was the spleen (Table I). This MHC-independent accumulation of splenic DC was the same for MLN-derived DC, suggesting a real predilection of DC for the spleen rather than their return to the tissue of origin.

TABLE V

Tissue Localization of T Lymphocytes (T), Glutaraldehyde-fixed Dendritic Cells (gDC) and Cell-free ^{111}Indium (^{111}In) after Administration into the Foodpad

Tissue	Radioactivity per organ (percent total dose injected)					
	T		gDC		^{111}In	
	3 h	24 h	3 h	24 h	3 h	24 h
Popliteal LN	0.23 (12.98)*	0.78 (124.06)	0.06 (5.61)	0.12 (19.4)	0.11 (9.0)	0.12 (14.2)
Inguinal LN	0	0.02	0	0.02	0.02	0.03
Para-aortic LN	0.04	0.13	0	0.04	0.04	0.05
MLN	0.03	0.05	0.03	0.03	0.07	0.04
Peyer's	0.01	0.02	0.01	0.03	0.04	0.04
Gut	0.58	1.62	0.69	1.29	2.26	2.95
Liver	1.15	3.47	1.31	2.49	3.57	5.53
Spleen	0.1	0.22	0.11	0.14	0.29	0.34
Lungs	0.17	0.29	0.24	0.15	0.65	0.32
Kidney	1.97	6.09	2.47	3.67	7.04	6.35
Skin	1.8	2.35	2.44	2.66	5.2	5.26
Hind feet	69.43	28.77	33.0	14.99	16.84	11.63
Blood	1.42	0.67	2.26	0.31	4.91	0.44
Total	76.93	44.48	42.62	25.94	41.04	33.1
n	3	5	3	2	2	3

For details see Materials and Methods and Table I legend.

* The values in parentheses represent the activity as a percentage of the total label recovered per 0.1 g of tissue.

A large number of DC migrated to the liver, the second major site of sequestration in terms of specific activity (Table I). T cells also migrated to the spleen and liver, but the relative amount of activity in these sites was opposite to that after transfer of DC (Table II). Using techniques that are described in the accompanying paper (17) to visualize the DC within frozen sections of liver, many appeared to localize in the sinusoids. Although we cannot exclude the possibility that at least some of these are dead or damaged cells, there are precedents for normal cellular migration from the liver. For example, the hepatic lymph has a higher flow rate and conveys a greater number of lymphocytes than any other peripheral lymph in the sheep (33). Moreover, S. Fossum (personal communication) using different techniques has shown that DC can migrate from the blood to the liver and thence to the coeliac lymph nodes which we did not examine.

The present studies suggest that an equilibrium exists between liver- and spleen-seeking DC, akin to that between spleen- and lymph node–seeking T cells (29). Thus, the number of DC entering the liver was inversely related to that migrating to the spleen in nude and T cell–reconstituted nude mice (Table III), and in splenectomized compared with normal mice. A similar equilibrium may exist for specifically sensitized T lymphocytes: after splenectomy of rats that were rejecting cardiac allografts, sensitized splenocytes that would otherwise have migrated to the spleen were sequestered in high numbers in the liver (34).

DC did not have access to Peyer's patches and MLN or other peripheral lymph

nodes from the blood, even after splenectomy. Apparently, DC lack some cell membrane constituent or receptor that would allow them to interact with the postcapillary high endothelial venules. In contrast, T cells homed readily to these sites (Table II) and there was increased entry into lymph nodes of splenectomized mice. This is compatible with the demonstration of DC in the afferent but not efferent or central lymph and is confirmatory evidence that, unlike T cells, DC do not recirculate from blood to lymph. In this respect, DC behave similarly to NK cells (35), peritoneal exudate macrophages (36), and splenic marginal zone B cells (37), which are unable to contribute to the cellular recirculating pool.

The radioactivity in other tissues after intravenous administration of viable DC was not detectably above control levels (Table I; compare legends to Tables I and II, and Tables IV and V). DC were rapidly cleared from the blood and the small amount of activity present at later times was in plasma rather than the cells (not shown); the levels in the kidney were probably related to the amount of isotope being excreted. Although the activity in the gut and skin was higher after transfer of DC than after T cells (compare Tables I and II) this was not above DC control levels. Either an undetectable number of DC entered these sites, thought to be important for differentiation of cells of the DC lineage, or another precursor in blood (for which there is as yet no evidence) gives rise to the Langerhans' cells and other DC-like cells of nonlymphoid tissues. DC isolated from peripheral blood of humans are indistinguishable in phenotype and function from those isolated from spleens and tonsils (15, 16). Presumably, they also exist in the peripheral blood of rodents, but for practical reasons they cannot be isolated. Perhaps this is the normal migratory and circulating form that is produced by the bone marrow, i.e., DC may be released mature (like B cells) rather than as immature precursors (like monocytes).

T Cell–dependent and –independent Migration of DC to Lymphoid Tissues. When the traffic of DC from the blood was examined in nude mice, we found they did not enter the spleen (Table III). However, when the nude mice were reconstituted with T cells the levels of DC in the spleen now approached those in euthymic mice. Thus migration of DC from the blood to the spleen is regulated by T cells. Although we initially thought that this might be due to direct DC–T cell clustering, analogous to that studied in vitro (30) and implicated in vivo (38), elsewhere we present evidence that is contrary to this idea (17). In particular, we have found that the DC are initially localized within the red pulp of the spleen but that later, and only after a period of what appears to be active migration, they enter the white pulp. Moreover, DC can bind to the marginal zone, perhaps to the endothelium, and it seems likely that this interaction may be the one that is controlled by the T cells (17).

We also examined migration of DC via the afferent lymphatics. DC injected SC into the footpad entered the draining popliteal lymph nodes (Table IV). Since they did not have access to lymph nodes from the blood (Table I), their sole route of entry may be from afferent lymph. Other observations (39) that macrophages became depleted from nodes that were surgically deprived of their afferent lymph supply may be relevant in this context. Furthermore, at least within 24 h, DC did not migrate beyond the local popliteal lymph nodes to the inguinal nodes, an observation consistent with the absence of DC in the efferent

lymph (12). There was no difference between euthymic and nude mice (Table IV), showing that migration into the node is independent of the presence of T cells.

That T cells can regulate the entry of DC into the spleen, but not lymph nodes, may have a bearing on the migration pathways of DC from the blood into nonlymphoid compartments. The present studies imply that DC, like cells of the mononuclear phagocyte series, may exhibit both constitutive and T cell–regulated migration into the tissues; monocytes, for instance, constitutively give rise to tissue macrophages in the steady state, but can also be recruited into sites of infection and inflammation (e.g., reference 40). We hypothesize that DC in blood could be important for the generation of local immune responses at extralymphoid sites, and are currently investigating whether DC accumulate at sites of inflammation and in organ allografts.

Summary

Dendritic cells (DC) are critical accessory cells for primary immune responses and they may be important stimulators of transplantation reactions, but little is known of their traffic into the tissues. We have studied the migration of purified splenic DC and T lymphocytes, labeled with ^{111}Indium-tropolone, in syngeneic and allogeneic mice.

First we demonstrate that DC can migrate from the blood into some lymphoid and nonlymphoid tissues. Immediately after intravenous administration, radiolabeled DC were sequestered in the lungs, but they actively migrated into the liver and spleen and reached equilibrium levels between 3 and 24 h after transfer. At least half of the radiolabel accumulated in the liver, but the spleen was the principal site of DC localization in terms of specific activity (radiolabel per weight of tissue). DC were unable to enter Peyer's patches, or mesenteric and other peripheral lymph nodes from the bloodstream. This was also true in splenectomized recipients, where the otherwise spleen-seeking DC were quantitatively diverted to the liver. In contrast, T cells homed readily to the spleen and lymph nodes of normal mice and increased numbers were present in these tissues in splenectomized mice. Thus, unlike T cells, DC cannot recirculate from blood to lymph via the nodes.

We then show that migration of DC from the blood into the spleen is dependent on the presence of T cells: DC did not enter the spleens of nude mice, but when they were reconstituted with T cells the numbers entering the spleen resembled those in euthymic mice. In nude mice, as in splenectomized recipients, the DC that would normally enter the spleen were quantitatively diverted to the liver. These findings suggest that there is a spleen–liver equilibrium for DC, that may be akin to that existing between spleen and lymph node for T cells.

Finally, we followed the traffic of radiolabeled DC via the afferent lymphatics after subcutaneous footpad inoculation. DC accumulated in the popliteal nodes but did not migrate further to the inguinal nodes. There was no difference between euthymic and nude mice, showing that unlike traffic to the spleen, this route probably does not require T cells. These migration patterns were not affected by major histocompatibility barriers, and were only seen with viable, but not glutaraldehyde-fixed, DC.

The demonstration of a novel migratory pathway for DC from the blood, together with T-dependent and -independent routes for entry to lymphoid tissues, has important implications for the traffic of DC from the blood into nonlymphoid tissues during inflammatory reactions and perhaps for the generation of extralymphoid immune responses.

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